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Condensation-cyclodehydration of 2,4-dioxobutanoates: Synthesis of new esters of pyrazoles and isoxazoles and their antimicrobial screening

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ABSTRACT

*A series of new substituted/unsubstituted pyrazole-3-carboxylates **(2-3a-d)** and isoxazole-3-carboxylates **(4a-d)** were synthesised by the reaction of methyl 4-(substituted/unsubstituted benzofuran-2-yl)-2,4-dioxobutanoates **(1a-d)** with active nitrogen-centered nucleophiles (hydrazine hydrate, hydroxylamine hydrochloride and semicarbazide hydrochloride) involving condensation-cyclodehydration of 2,4-dioxobutanoates. Constitutions of the synthesised compounds have been delineated on the basis of chemical transformations, elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. The title compounds were evaluated for their antimicrobial activity against gram positive and gram negative bacteria as well as a fungus.*

Key words: 2,4-Dioxobutanoate, pyrazoles-3-carboxylate, isoxazole-3-carboxylate

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INTRODUCTION

Benzofuran derivatives are an important class of heterocyclic compounds that have attracted much attention over the last few years because of their profound physiological and chemotherapeutic properties and their widespread occurrence in nature [1]. Synthesis and reactions of various benzofurans, 2,4-dioxobutanoates, isoxazoles, pyrazoles and its carboxylate derivatives have been reported earlier. Among the various routes for the syntheses of pyrazoles, the most convenient is the condensation of 1,3-diketones with hydrazine or substituted hydrazine [2,3]. Many synthetic methods have been employed in the preparation of isoxazoles [4,5]. Scores of references are available in literature which reveals that pyrazoles and their related fused heterocycles have attracted much attention of organic chemist due to their chemotherapeutic and biological activities such as anticancer [6], analgesic and antipyretic [7], bacteriostatic, bactericidal, fungicidal and antimalarial [8]. Similarly, derivatives of isoxazole constitute as an important heterocycle in view of their use as analgesic, fungicides, antinflammmatory, ulcerogenic and anticancer activities [9]. Due to the presence of reactive methylene group, the 1,3-dicarbonyl part of the diketoester can be easily and efficiently used as a synthon for the synthesis of new nitrogen and oxygen heterocycles with various organic reagents. This prompted us to explore the possibility of the use of 2,4-dioxobutanoates bearing benzofuran moiety in the synthesis of new pyrazoles and isoxazoles with a carboxylate group as one of the substituents for the synthesis of polycyclic systems containing bridgehead nitrogen and oxygen heterocycles by using the reference procedures [10,11] and to investigate their biological activities.

MATERIALS AND METHODS

The melting points were recorded in open capillary in paraffin bath and are uncorrected. IR spectra were recorded on a Shimadzu IR Spectrophotometer (KBr, v max in cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra are recorded on a Bruker AM 400 instrument (400 MHz) using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 and $CDCl_3$ as solvent. Chemical Shifts are given in parts per million (ppm). Positive-ion electrospray ionisation (ESI) mass spectra

were obtained with a Waters Micromass Q–TOF Micro, Mass Spectrophotometer. Elemental analysis (CHN) was done using Elemental analyzer, Vario EL III. All the chemicals used for the synthesis were of AR grade of Merck, S.D.Fine and Aldrich. The reactions were monitored by E.Merck TLC aluminum sheet silica $gel_{60}F_{254}$ and visualizing the spot in UV Cabinet and iodine chamber. The compounds were analyzed for carbon, hydrogen and nitrogen the results were in good agreement with the calculated values.

Experimental Procedure

General procedure for the synthesis of methyl 4-(substituted/unsubstituted benzofuran-2-yl)-2,4-dioxobutanoates (1a-d) [12]**: 1a-d** were prepared by adopting the published reference method in the literature.

General procedure for the synthesis of methyl 5-(substituted/unsubstituted benzofuran-2-yl)-1*H*-pyrazole-3-carboxylates (2a-d): To a mixture of **1a** (10 mmol) in acetic acid (10mL), hydrazine hydrate (30 mmol) was added gradually with constant stirring and refluxed for 2h. Then it was poured in ice cold water, filtered, washed, dried and further purified by recrystallization by ethanol to obtain **2a**. Similarly, **2b-d** were synthesised from **1b-d** by adopting the same procedure followed for **2a** and their structural ability further proved by spectral and analytical techniques.
Methyl 5-(benzofuran-2-yl)-1*H*-pyrazole-3-carboxylate (2a): White crystalline solid; mp 180-182 ˚C; yield 90%; M. F. $C_{13}H_{10}O_3N_2$; Calculated: C, 64.46; H, 4.13; N, 11.57 Found: C, 64.21; H, 4.34; N, 11.77. [13]

Methyl 5-(5-bromobenzo furan-2-yl)-1*H*-pyrazole-3-carboxylate (2b): White crystalline solid; mp 221-223˚C; yield 85%; M. F. $C_{13}H_9O_3N_2Br$; IR: 3263, 3153 (NH), 3049 (ArH), 2945 (CH_3), 1690 (C=O), 1595 (C=N),1451, 1445 (C=C), 1270(C-O-C); ^{1}H NMR: 3.6463 (s, 3H, -COOC$\underline{H}_3$), 7.6130-7.8622 (m, 6H, ArH + NH); ^{13}C NMR: 53(CH_3O), 105, 110, 116, 120, 123, 125, 129, 130, 132, 140(Ar C_1-C_{10}), 155(C-O), 169(C=O) MS: *m/z* 322 [M +H]$^+$, 344 [M+Na]$^+$ Calculated: C, 48.59; H, 2.80; N, 8.72 Found: C, 48.72; H, 2.75; N, 8.88

Methyl 5-(5-chloro-3-methyl benzofuran-2-yl)-1*H*-pyrazole-3-carboxylate (2c): White crystalline solid; mp 208-210˚C; yield 81%; M. F. $C_{14}H_{11}O_3N_2Cl$; IR: 3290, 3148 (NH), 3039 (ArH), 2950 (CH_3), 1694 (C=O), 1573 (C=N), 1485, 1439 (C=C), 1266 (C-O-C); ^{1}H NMR: 2.1234 (s, 3H, CH_3), 3.8754 (s, 3H, -$COOCH_3$), 6.3222 (s, 1H, pyrazole CH), 7.2443-7.4577 (m, 4H, ArH) Calculated: C, 57.93; H, 3.79; N, 9.65 Found: C, 57.58; H, 3.62; N, 9.64
Methyl 5-(5,7-dichloro-3-methylbenzo furan-2-yl)-1*H*-pyrazole-3-carboxylate (2d): White crystalline solid; mp 222-224 ˚C; yield 80%; M. F. $C_{13}H_{10}O_3N_2Cl_2$; IR: 3290, 3148 (NH), 3039 (ArH), 2995 (CH_3), 1720 (C=O), 1590 (C=N), 1485, 1439 (C=C), 1265 (C-O-C); ^{1}H NMR: 2.1824 (s, 3H, CH_3), 3.9336 (s, 3H, -$COOCH_3$), 6.1534 (s, 1H, pyrazole CH), 7.2204-7.4705 (m, 3H, ArH); Calculated: C, 48; H, 3.08; N, 8.62 Found: C, 47.92; H, 3.11; N, 8.59

General procedure for the synthesis of methyl 5-(substituted/unsubstituted benzofuran-2-yl)-1-carbamoyl-1*H*-pyrazole-3-carboxylates (3a-d): To a mixture of **1a** (5 mmol) in ethanol (10mL), semicarbazide hydrochloride (10 mmol) and sodium acetate (10 mmol) were added; the reaction mixture was refluxed for 4h. It was then concentrated, cooled and poured in ice cold water; solid separated out was filtered, washed, dried and further purified by recrystallization using ethanol to give **3a**. Similarly, **3b-d** were synthesised from **1b-d** by extending the same procedure followed for **3a** and their structural identities were proved by spectral and analytical techniques

Methyl 5-(benzofuran-2-yl)-1-carbamoyl-1*H*-pyrazole-3-carboxylate (3a): White crystalline solid; mp 142-144 ˚C; yield 76%; M. F. $C_{14}H_{11}O_4N_3$; Calculated: C, 58.94; H, 3.85; N, 14.73 Found: C, 58.88; H, 3.68; N, 14.54. [13]

Methyl 5-(5-bromobenzofuran-2-yl)-1-carbamoyl-1*H*-pyrazole-3-carboxylate (3b): White crystalline solid; mp 190-192˚C; yield 68%; M. F. $C_{14}H_{10}O_4N_3Br$; IR: 3456, 3410, 3258, 3156 (NH_2), 3032 (ArH), 2955(CH_3), 1722 (C=O), 1690 (C=O), 1599 (C=N), 1375 (C-N), 1274 (C-O-C); ^{1}H NMR: 3.9531 (s, 3H,-COOC$\underline{H}_3$), 6.1344 (s, 1H, pyrazole CH), 7.2583-7.8585 (m, 6H, ArH); Calculated: C, 46.15; H, 2. 74; N, 11.54 Found: C, 46.45; H, 2.89; N, 11.62

Methyl 5-(5-chloro-3-methylbenzofuran-2-yl)-1-carbamoyl-1*H*-pyrazole-3-carboxylate (3c): White crystalline solid; mp 169-171˚C; yield 70%; M. F. $C_{15}H_{12}O_4N_3Cl$; IR: 3460, 3408, 3253, 3160 (NH_2), 3045 (ArH), 2943 (CH_3), 1740 (C=O), 1692 (C=O), 1578 (C=N), 1376 (C-N), 1256 (C-O-C); ^{1}H NMR: 2.3024 (s, 3H, CH_3), 3.7531 (s, 3H,-COOC$\underline{H}_3$), 6.3110 (s, 1H, pyrazole CH), 7.2339-7.7623 (m, 5H, ArH); ^{13}C NMR: 8(CH_3), 50(CH_3O), 105, 109, 112, 120, 121, 124, 125, 135(Ar C_1-C_8), 150, 152(C-O), 152, 165(C=O); Calculated: C, 54.05; H, 3.60; N, 12.61 Found: C, 53.99; H, 3.88; N, 12.98

Methyl 5-(5,7-dichloro-3-methylbenzofuran-2-yl)-1-carbamoyl-1*H*-pyrazole-3-carboxylate (3d): White crystalline solid; mp 144-146 ˚C; yield 65%; M. F. $C_{15}H_{11}O_4N_3Cl_2$
Calculated: C, 49.04; H, 2.99; N, 11.44 Found: C, 49.00; H, 3.00; N, 11.38

General procedure for the synthesis of ethyl 5-(substituted/unsubstituted benzofuran-2-yl)-isoxazole-3-carboxylates (4a-d): To a mixture of **1a** (10 mmol) in ethanol (200 mL), hydroxylamine hydrochloride (20 mmol) and sodium acetate (20 mmol) were added, the reaction mixture was refluxed for 4h, kept overnight, solid obtained was then refluxed for 2h in ethanol (50mL) by adding conc. HCl (1mL). Excess of the solvent was evaporated, cooled, filtered and washed with water and further purified by recrystallization using ethanol to give **4a**. Similarly, **4b-d** were synthesised from **1b-d** by extending the same procedure followed for **4a** and their structural identities were further proved by spectral and analytical techniques.

Ethyl 5-(benzofuran-2-yl)-isoxazole-3-carboxylate (4a): White crystalline solid; mp 80-82 °C; yield 90%; M. F. $C_{14}H_{11}O_4N$; Calculated: C, 65.36; H, 4.28; N, 5.45 Found: C, 65.03; H, 4.01; N, 5.39 [13]

Ethyl 5-(5-bromobenzofuran-2-yl)-isoxazole-3-carboxylate (4b): Pale yellow crystalline solid; mp 135-137°C; yield 88%; M. F. $C_{14}H_{10}O_4NBr$; IR: 3032 (ArH), 2975(CH_3), 1720 (C=O), 1599 (C=N), 1477, 1459, 1435(C=C), 1260 (C-O-C); ^{1}H NMR: 1.4407-1.4763 (t, *J* = 7.12 Hz, 3H, -OCH$_2$<u>CH$_3$</u>), 4.2361-4.2895 (q, *J* = 7.12 Hz, 2H, -O<u>CH$_2$</u> CH$_3$), 7.2395-7.7501 (m, 5H, ArH); ^{13}C NMR: 15 (CH_3), 60 (CH_2O-), 102, 105, 110, 121, 122, 125, 127, 141 (Ar C_1-C_8), 152, 155, 156(C-O), 169(C=O); MS: *m/z* 337 $[M+H]^+$, 359 $[M+Na]^+$; Calculated: C, 50.00; H, 2.97; N, 4.17 Found: C, 49.97; H, 2.97; N, 4.10

Ethyl 5-(5-chloro-3-methylbenzofuran-2-yl)-isoxazole-3-carboxylate (4c): Pale yellow crystalline solid; mp 168-170°C; yield 88%; M. F. $C_{15}H_{12}O_4NCl$; IR: 3065 (ArH), 2975, 2954 (CH_3), 1735 (C=O), 1599 (C=N), 1465, 1448(C=C), 1266 (C-O-C); ^{1}H NMR: 2.2173 (s, 3H, CH_3), 1.4105-1.4462 (t, *J* = 7.16 Hz, 3H, -OCH$_2$<u>CH$_3$</u>), 4.2860-4.3397 (q, *J* = 7.16 Hz, 2H, -O<u>CH$_2$</u> CH$_3$), 7.2124-7.6743 (m, 4H, ArH); Calculated: C, 59.02; H, 3.93; N, 4. 59 Found: C, 58.98; H, 3.58; N, 4.55

Ethyl 5-(5,7-dichloro-3-methylbenzofuran-2-yl)-isoxazole-3-carboxylate (4d): Pale yellow crystalline solid; mp 162-165 °C; yield 85%; M. F. $C_{15}H_{11}O_4NCl_2$; ^{1}H NMR: 2.2231 (s, 3H, CH_3), 1.4035-1.4391 (t, *J* = 7.12 Hz, 3H, -OCH$_2$<u>CH$_3$</u>), 4.2660-4.3194 (q, *J* = 7.12 Hz, 2H, -O<u>CH$_2$</u> CH$_3$), 7.2124-7.6743 (m, 3H, ArH); Calculated: C, 53.09; H, 3.24; N, 4.13 Found: C, 53.15; H, 3.20; N, 4.28

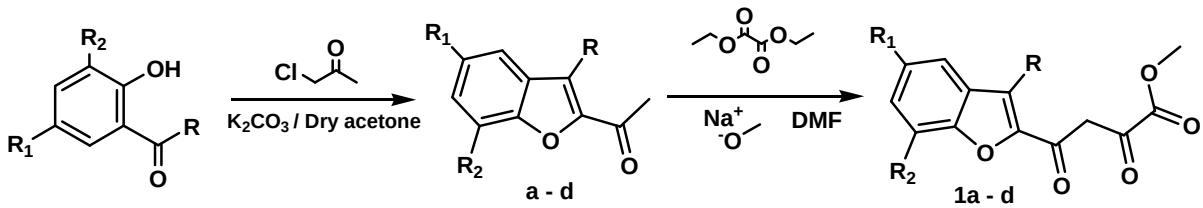
Antimicrobial Activity

All the novel synthesized compounds from **2-4a-d** have been screened for their *in vitro* antibacterial activity against two gram positive strains i.e. *Bacillus subtilis* (NCIM 2439) and *Staphylococcus aureus* (NCIM 2079) and two gram negative strains i.e. *Escherichia coli* (NCIM 2064) and *Pseudomonas aeruginosa* (NCIB 8650) by using Mueller Hinton Agar and antifungal activity against a fungus *Aspergillus niger* (NCIM 501) using Sabouraud Dextrose agar using cup plate agar diffusion method [14] by measuring the inhibition zone in mm. The compounds were taken at a concentration of 1mg/mL using Dimethyl Sulphoxide (DMSO) as negative control. Chloramphenicol was used as standard for antibacterial and Kanamycin for antifungal activity. The plates were incubated at 37°C for 24 hours for bacteria and 28°C for 72-96 hours for fungus. Zone of inhibition observed around the cup after respective incubation was measured in four directions with the help of Vernier Calipers. The inhibitory effects of the synthesised compounds are summarized in the Table **1**.

RESULTS AND DISCUSSION

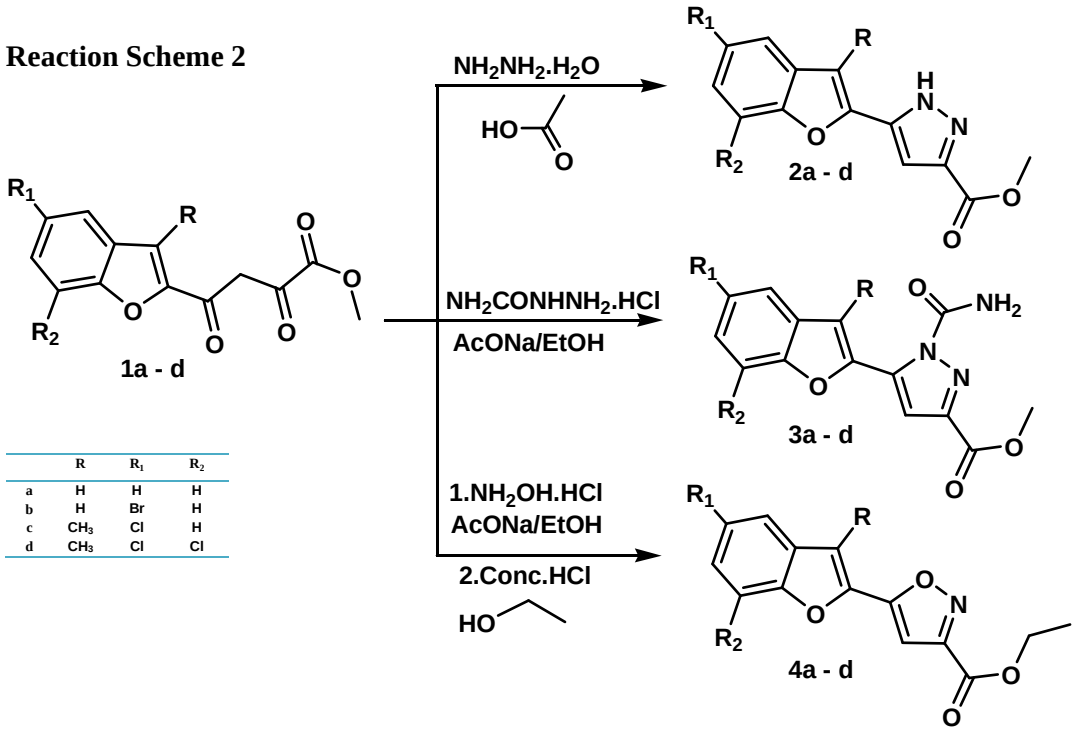
Synthesis of methyl 4-(substituted/unsubstituted benzofuran-2-yl)-2,4-dioxobutanoates **(1a-d)** by the condensation of appropriate hetaryl ketones **a-d** and diethyl oxalate in presence of sodium metal in methanol was carried out in good yields as shown in reaction scheme **1** [12].

Reaction Scheme 1



The synthesis of the novel compounds from **2a-d** to **4a-d** is described in reaction scheme **2**.The reactions were monitored by TLC. The identities of the newly synthesized compound have been established on the basis of their elemental analysis and spectral data [15] such as IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. The analytical data of the compounds are mentioned. These compounds were screened for their antimicrobial activities.

Reaction Scheme 2



	R	R_1	R_2
a	H	H	H
b	H	Br	H
c	CH_3	Cl	H
d	CH_3	Cl	Cl

Reaction of **1a-d** with hydrazine hydrate in acetic acid afforded **2a-d**. The IR spectrum of **2b** showed a characteristic NH stretch at 3263 and 3153 cm^{-1} is in evidence of closure of pyrazole ring. The spectral data including IR, ^1H NMR and ^{13}C NMR similarly, its mass spectra revealed a $[M+H]^+$ peak at 322, which is in agreement with the molecular formula $C_{13}H_9O_3N_2Br$. Treatment of **1a-d** with semicarbazide hydrochloride and sodium acetate in ethanol yielded **3a-d**. **3c** exhibited IR stretch at 3460 and 1376 cm^{-1} due to NH_2 and C-N of -$CONH_2$ group respectively. Similarly, IR, ^1H NMR and ^{13}C NMR spectral data also confirmed its structure. Reaction of **1a-d** with hydroxyl amine hydrochloride and sodium acetate in ethanol followed by concentrated HCl furnished **4a-d.** The signal at 1599 cm^{-1} in IR spectra due to C=N stretch confirms formation of isoxazole ring. ^1H NMR spectra of **4b,** gave a quartet at δ 4.2361-4.2895 and a triplet at δ 1.4407-1.4763 ppm due to the presence of $–COOCH_2CH_3$, confirmed that transesterification has also occurred simultaneously.

All the synthesised compounds gave deep red or violet red colouration in response to the hydroxamic test for ester, confirming their formation. Formulation of these reaction products was based upon the comparative reactivity of two carbonyl group in **1a-d** the C_2 carbonyl group being more reactive than C_4 carbonyl group, it gets preferably attacked by the nucleophilic reagents like hydrazine hydrate and semicarbazide hydrochloride to give corresponding hydrazone intermediate which simultaneously undergo ring closure with elimination of water molecule from imino proton of hydrazone residue and the –OH group of enolized C_4 carbonyl group to form the pyrazole ring.

Table 1 – Inhibition zone in (mm) of the compounds 2-4a-d

Compound	*Antibacterial*				*Antifungal*
	B. subtilis (NCIM 2439)	*S. aureus* (NCIM 2079)	*E. coli* (NCIM 2064)	*P. aeruginosa* (NCIB 8650)	*A. niger* (NCIM 501)
2a	10	12	11	11	18
2b	14	16	18	20	21
2c	24	29	16	23	21
2d	27	30	19	23	23
3a	-	-	10	10	20
3b	15	18	19	23	17
3c	25	25	18	21	20
3d	24	26	19	21	19
4a	09	12	10	14	18
4b	15	13	12	11	18
4c	23	28	14	12	20
4d	24	28	15	17	22
Kanamycin	-	-	-	-	23
Chloramphenicol	29	32	21	25	-
DMSO	-	-	-	-	-

Antimicrobial Activity

The results indicate that the synthesized compounds showed moderate to good activity against the selected strains (Table **1**). Compounds **2c, 2d, 3c, 3d, 4c** and **4d** showed good activity against *B. subtilis* and *S. aureus*, **2b, 2c, 2d, 3b, 3c and 3d** showed good activity against *E. coli*, and *P. aeruginosa*. While rest of the compounds are found to be poor or inactive against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. Similarly, all the synthesized compounds showed strong activity against the fungus, *A. niger*.

CONCLUSION

In conclusion, we have reported herein synthesis of some new heterocyclic esters such as pyrazole-3-carboxylates **(2,3a-d)**, and isoxazole-3-carboxylates **(4a-d)** from methyl 4-(substituted/unsubstituted benzofuran-2-yl)-2,4-dioxobutanoates **(1a-b)**. Antimicrobial screening of the synthesized compounds was done and found to possess moderate to good activity against selected strains of bacteria and fungus.

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